

## Claims

### What is claimed is:

- 5 1. A method of producing a heterologous biological substance, comprising:
  - (a) cultivating a mutant of a parent *Bacillus* cell in a medium suitable for the production of a heterologous biological substance, wherein (i) the mutant cell comprises a first nucleic acid sequence directing synthesis of the heterologous biological substance and a second nucleic acid sequence comprising a modification of at least one of the genes *cypX* and  
10 *yvmC*, which are involved in the production of a red pigment, and (ii) the mutant cell is deficient in the production of the red pigment compared to the parent *Bacillus* cell when cultivated under the same conditions; and
  - (b) recovering the heterologous biological substance from the cultivation medium.
- 15 2. The method of claim 1, wherein at least one gene of the second nucleic acid sequence is *cypX*.
3. The method of claim 1, wherein at least one gene of the second nucleic acid sequence is *yvmC*.
- 20 4. The method of claim 1, wherein the biological substance encoded by the first nucleic acid sequence is a biopolymer.
5. The method of claim 4, wherein the biopolymer is selected from the group consisting of  
25 a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.
6. The method of claim 5, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter protein, structural protein, and transcription factor.
- 30 7. The method of claim 6, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

8. The method of claim 5, wherein the polysaccharide is chitin, heparin, hyaluronan, or hyaluronic acid.

9. The method of claim 1, wherein the biological substance encoded by the first nucleic acid sequence is a metabolite.

10. The method of claim 1, wherein the first nucleic acid sequence comprises a biosynthetic or metabolic pathway.

11. The method of claim 1, which the mutant cell comprises at least two copies of the first nucleic acid sequence directing synthesis of a biological substance.

12. The method of claim 1, wherein the *Bacillus* cell is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* cell.

13. The method of claim 1, wherein the *Bacillus* cell is a *Bacillus subtilis* cell.

14. The method of claim 1, wherein the *Bacillus* cell is a *Bacillus licheniformis* cell.

15. The method of claim 1, wherein the mutant cell produces at least about 25% less of the red pigment compared to the parent *Bacillus* cell when cultured under identical conditions.

16. The method of claim 1, wherein the mutant cell produces no detectable red pigment compared to the parent *Bacillus* cell when cultured under identical conditions.

17. The method of claim 1, wherein the mutant cell further comprises a modification of one or more genes which encode a protease.

18. The method of claim 17, wherein the genes are *nprE* and/or *aprE*.

19. The method of claim 1, wherein the mutant cell further comprises a modification of one

or more genes selected from the group consisting of *spollAC*, *srfA*, *srfB*, *srfC*, *srfD*, and *amyE* genes.

20. A mutant of a parent *Bacillus* cell, comprising a first nucleic acid sequence directing synthesis of a heterologous biological substance and a second nucleic acid sequence comprising a modification of at least one of the genes *cypX* and *yvmC*, which are involved in the production of a red pigment, wherein the mutant cell is deficient in the production of the red pigment compared to the parent *Bacillus* cell when cultivated under the same conditions.

21. The mutant cell of claim 20, wherein at least one gene of the second nucleic acid sequence is *cypX*.

22. The mutant cell of claim 20, wherein at least one gene of the second nucleic acid sequence is *yvmC*.

23. The mutant cell of claim 20, wherein the biological substance encoded by the first nucleic acid sequence is a biopolymer.

24. The mutant cell of claim 23, wherein the biopolymer is selected from the group consisting of a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.

25. The mutant cell of claim 24, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter protein, structural protein, and transcription factor.

26. The mutant cell of claim 25, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

27. The mutant cell of claim 24, wherein the polysaccharide is chitin, heparin, hyaluronan, or hyaluronic acid.

28. The mutant cell of claim 20, wherein the biological substance encoded by the first nucleic acid sequence is a metabolite.

29. The mutant cell of claim 20, wherein the first nucleic acid sequence comprises a biosynthetic or metabolic pathway.

30. The mutant cell of claim 20, which comprises at least two copies of the first nucleic acid sequence directing synthesis of a biological substance.

31. The mutant cell of claim 20, wherein the *Bacillus* cell is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* cell.

32. The mutant cell of claim 20, which is a *Bacillus subtilis* cell.

33. The mutant cell of claim 20, which is a *Bacillus licheniformis* cell.

34. The mutant cell of claim 20, which produces at least about 25% less of the red pigment compared to the parent *Bacillus* cell when cultured under identical conditions.

35. The mutant cell of claim 20, which produces no detectable red pigment compared to the parent *Bacillus* cell when cultured under identical conditions.

36. The mutant cell of claim 20, which further comprises a modification of one or more genes which encode a protease.

37. The mutant cell of claim 36, wherein the genes are *nprE* and/or *aprE*.

38. The mutant cell of claim 20, which further comprises a modification of one or more genes selected from the group consisting of *spolIAC*, *srfA*, *srfB*, *srfC*, *srfD*, and *amyE* genes.

39. A method of obtaining a mutant of a parent *Bacillus* cell, comprising:

(a) introducing into the parent *Bacillus* cell a first nucleic acid sequence directing synthesis of a heterologous biological substance and a second nucleic acid sequence

comprising comprising a modification of at least one of the genes *cypX* and *yvmC*, which are involved in the production of a red pigment; and

(b) identifying the mutant cell from step (a) comprising the modified nucleic acid sequence, wherein the mutant cell is deficient in the production of the red pigment compared to the parent *Bacillus* cell when cultivated under the same conditions.

40. The method of claim 39, wherein at least one gene of the second nucleic acid sequence is *cypX*.

41. The method of claim 39, wherein at least one gene of the second nucleic acid sequence is *yvmC*.

42. The method of claim 39, wherein the biological substance encoded by the first nucleic acid sequence is a biopolymer.

43. The method of claim 42, wherein the biopolymer is selected from the group consisting of a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.

44. The method of claim 43, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter protein, structural protein, and transcription factor.

45. The method of claim 44, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

46. The method of claim 43, wherein the polysaccharide is chitin, heparin, hyaluronan, or hyaluronic acid.

47. The method of claim 39, wherein the biological substance encoded by the first nucleic acid sequence is a metabolite.

48. The method of claim 39, wherein the first nucleic acid sequence comprises a biosynthetic or metabolic pathway.

49. The method of claim 39, which the mutant cell comprises at least two copies of the first nucleic acid sequence directing synthesis of a biological substance.

50. The method of claim 39, wherein the *Bacillus* cell is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* cell.

51. The method of claim 39, wherein the *Bacillus* cell is a *Bacillus subtilis* cell.

52. The method of claim 39, wherein the *Bacillus* cell is a *Bacillus licheniformis* cell.

53. The method of claim 39, wherein the mutant cell produces at least about 25% less of the red pigment than the parent *Bacillus* cell when cultured under identical conditions.

54. The method of claim 39, wherein the mutant cell produces no detectable red pigment compared to the parent *Bacillus* cell when cultured under identical conditions.

55. The method of claim 39, wherein the mutant cell further comprises a modification of one or more genes which encode a protease.

56. The method of claim 55, wherein the genes are *nprE* and/or *aprE*.

57. The method of claim 39, wherein the mutant cell further comprises a modification of one or more genes selected from the group consisting of *spolIAC*, *srfA*, *srfB*, *srfC*, *srfD*, and *amyE* genes.